prone to aggregation. In this study, to overcome this issue and chemically regulate disulfide-coupled peptide and protein folding, a series of chemical reagents was examined in the refolding of hepcidin and prohepcidin, as a model peptide and protein, and their folding recoveries were estimated.
Hepcidin consists of 25 amino acid residues and four intra-molecular disulfide bonds, which are absolutely required for its biological activity [2], not only for iron homeostasis, but also for anti-microbial activity. To investigate structure-function relationships, hepcidin was chemically synthesized. However, the yield of synthesized hepcidin was quite low under the typical folding conditions. The major problem in the disulfide-coupled folding of hepcidin is that it undergoes aggregation during its folding reaction [3]. To solve this problem, several types of redox reagents and solvents were examined to improve the folding efficiency of hepcidin [3]. However, all of the reagents resulted in quite low yields for the disulfide-coupled folding of hepcidin. Therefore, to regulate the folding reaction of hepcidin and its precursor protein, we estimated the folding conditions, such as pH values, salt concentrations, and a variety of redox reagents. The results will be discussed in this paper.
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Spectroscopic and SAXS Studies of Human Cystatin C Mutants - Early Stages of Amyloid Formation Process
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Human cystatin C (HCC) is a cysteine protease inhibitor. This protein in pathological conditions, forms dimers via a ''domain swapping'' mechanism. HCC is also associated with two types of amyloid deposition diseases - hereditary amyloid angiopathy (related to the Leu68Gln mutation) and wild-type cystatin C co-precipitation.
The aim of our studies was the characterisation of the self-assembling properties of native and mutated (at positions 57 or 68) forms of human cystatin C in solution. The structure, overall conformation and secondary structure changes in solution were studied by Fourier transformed infrared spectroscopy (FTIR), circular dichroism spectroscopy (CD), dynamic light scattering (DLS) and time resolved small angle scattering of synchrotron radiation (TR-SAXS).
SAXS data for native and mutated HCC were subjected to analysis by using SVD and MCR-ALS methods as well as the low resolution structure determination. Besides the monomeric forms of human cystatin C, also dimers and higher oligomers were formed even after short (50-ms) exposure on synchrotron radiation. In addition we observed for first time, formation of domain swapped dimers of human cystatin C induced by irradiation. The spectroscopic studies confirmed conformational changes.
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Differential Effects on Light Chain Amyloid Formation Depend on Mutations and Type of Glycosaminoglycans
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Light chain (AL) amyloidosis is a protein misfolding disease where immunoglobulin light chains sample partially folded states that lead to misfolding and amyloid formation, resulting in organ dysfunction and death. In vivo, amyloid deposits are found in the extracellular space and involve a variety of accessory molecules such as glycosaminoglycans, one of the main components of the extracellular matrix. Glycosaminoglycans are a group of negatively charged, heteropolysaccharides composed of repeating disaccharide units. In this study, we investigated the effect of glycosaminoglycans on the kinetics of amyloid fibril formation of three AL cardiac amyloidosis light chains. These proteins have similar thermodynamic stability but exhibit different kinetics of fibril formation. We also studied single restorative and reciprocal mutants and wild type germline control protein.
We found that the type of glycosaminoglycan has different effect on the kinetics of fibril formation, and this effect seems to be associated with the natural propensity of each AL protein to form fibrils. Heparan sulfate accelerated AL-12, AL-09, κI Y87H, and AL-103 H92D fibril formation, it delayed fibril formation for AL-103 and did not promote any fibril formation for AL-12 R65S, AL-103 delP95aIns or κI O18/O8. Chondroitin sulfate A on the other hand, shows a strong fibril formation inhibition for all proteins.
We propose that Heparan sulfate facilitates the formation of transient amyloidogenic conformations of AL light chains, hereby promoting amyloid formation, whereas Chondroitin sulfate A kinetically traps partially unfolded intermediates and further fibril elongation into fibrils is inhibited, resulting in formation/accumulation of oligomeric/protofibrillar aggregates.

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Small Angle Neutron and X-Ray Scattering of Plasma Glycoprotein Interactions with Lipid Membranes
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Fibrinogen and Alpha 1-Antitrypsin (A1AT) are plasma glycoproteins with different, but specific functions. A1AT has been shown to have protective roles of lung cells against emphysema, while fibrinogen is a major factor in the blood clotting process. Most known glycoproteins have been shown to play a role in cellular interactions but the exact role of the glycan chains is still under investigation. Previous electrophysiological measurements show that A1AT has a strong affinity to lipid bilayers, perturbing the function of ion channels present in the membrane. These observed protein-membrane and protein-protein interactions in solution were studied using contrast-matching small-angle neutron scattering (SANS), small angle x-ray scattering (SAXS) and dynamic light scattering (DLS). To establish a structural reference point for each protein in solution, a series of polyethelene glycol-1,500 MW (PEG-1500) induced osmotic stress measurements were performed. The following D2O:H2O contrast matches were used for SANS: 40% for proteins, 15% for lipids and 16% for PEG-1500. Radius of gyration (Rg) approximations using Guinier analysis of A1AT in solution show a structural phase transition when the concentration of PEG-1500 is between 33% and 36% by volume. Significant structural changes were also observed for fibrinogen when the concentration of PEG-1500 was above 40% by volume. These structural changes were compared with changes observed when A1AT was in the presence of three different lipid membranes: POPC, POPS and DLPC and when fibrinogen undergoes polymerization. Bragg peaks produced by lipid membranes show that A1AT interacts with unilamellar vesicles. DLS was used to find suitable concentrations of fibrinogen (4 mg/ml) and thrombin (0.01 units/ml) that would yield a significant signal-to-noise ratio for SANS experiments (or approximately 90 minutes of reaction time). SANS time-resolved measurements show that fibrin structure is affected by its polymerization rate.

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Analytical Characterization of FGF Signaling Complex
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Fibroblast growth factors (FGF) are a family of growth hormones that act as regulators in key biological processes such as angiogenesis, wound healing, embryonic development and select endocrine signaling pathways. It is currently theorized that the mechanism of the interaction of FGF to their receptors, fibroblast growth factor receptors (FGFR) is greatly reliant upon the binding of heparin to FGFs. It is theorized that the D2 domain is the primary site for the FGF bind and interaction. The objective of this study is to characterize the structure of the FGF-D2 domain. A structural analog of heparin, sucrose octasulfate (SOS), is employed in this study to determine the role of heparin in the formation of the FGF-FGFR complex. Isothermal titration calorimetry experiments were preformed to assess the binding affinity of FGF and the D2 domain, these experiments indicate that human acidic FGF (FGF-1) binds to the D2 domain with high affinity in both the presence and absence of SOS ($K_{d(appa)} \sim 10^{-7}$ M and $K_{d(appa)} \sim 10^{-8}$ M respectively). Thermal denaturation experiments, monitored by far-UV CD, indicate that both FGF-1 and D2 domain undergo slight conformational changes as a result of binding and elucidates the presence of SOS stabilizes a 1:1 FGF-D2 domain complex. The FGF signaling complex, comprised of the FGF-1, the D2 domain, and SOS, structure elucidated by X-ray crystallography indicated that the complex exists as a 2:2:2 symmetrical conjugation. Analysis via 2D ^{1}H and ^{15}N NMR chemical shift perturbation (CSP) has allowed for the accurate mapping of the SOS and FGF binding sites of the D2 domain. The data resulting from this study suggest that the primary role of heparin in the FGF signaling process is limited to stabilizing the FGF-FGFR complex.